Data Evaluation Record on the Acute Dietary Toxicity of Acetochlor Technical to Zebra Finches, *Taeniopygia guttata*

PMRA Submission Number {.....} EPA MRID Number 51159004

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	459470
	OECD Data Point	{.....}
	EPA MRID	51159004
	EPA Guideline	850.2200

Test material: **Acetochlor Technical** **Purity:** 96.6%
Common name:
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No: Not reported
Synonyms: Not reported

Primary Reviewer: Elizabeth Krupka
Environmental Scientist, CDM/CSS-Dynamac JV
Signature:
Date: 10/28/2020

Secondary Reviewer: Adrian Graff
Environmental Scientist, CDM/CSS-Dynamac JV
Signature:
Date: 12/29/2020

Primary Reviewer: Meghann Niesen
EPA/EFED/ERB5
Digitally signed by MEGHANN NIESEN
Date: 2021.04.13 09:19:07 -04'00'

EPA PC Code 121601

Date Evaluation Completed: 04-13-2021

CITATION: Hubbard, B.S., Temple, D.L., Sneckenberger, G., and L. Zhang. 2020. Acetochlor Technical: A Dietary LC_{50} Study with the Zebra Finch. Study performed Eurofins EAG Agroscience, LLC, Easton, Maryland. Study number 662B-101. Study sponsored by Sharda Cropchem Ltd., Mumbai, India. Study initiated September 13, 2019 and completed January 7, 2020.

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

EXECUTIVE SUMMARY:

The acute dietary toxicity of **Acetochlor Technical** to young adult zebra finches (*Taeniopygia guttata*) was assessed over 8 days. The test substance was administered to the birds for 5 days in the diet at nominal concentrations of 0 (negative control), 218, 500, 1000, 2000, and 4000 mg ai/kg diet, followed by a 3-day recovery period with untreated feed. Reviewer-calculated mean-measured concentrations were <200 (<LOQ, control), 212, 468, 948, 1920, and 3419 mg ai/kg diet.

After 8 days, mortality was 0% in the control and the two lowest treatment groups. Mortality increased to 50% in the mean-measured 948 mg ai/kg diet treatment group and 100% in the 1920 and 3419 mg ai/kg diet treatment groups.

The 8-day dietary LC_{50} value was 948 mg ai/kg diet. Based on the results of this study, **Acetochlor Technical** would be classified as **moderately toxic** to young adult zebra finches (*Taeniopygia guttata*) in accordance with the classification system of the U.S. EPA.

The study author noted statistically significant reductions of mean body weight at the 948, 1920, and 3419 mg ai/kg diet test concentrations during the exposure period. No statistically significant reductions in food consumption were observed in any treatment group relative to the negative control group.

Gross necropsies conducted on all mortalities resulted in observations of empty gastro-intestinal tracts, pale kidneys, mottled liver, pale liver, and pale spleens. No remarkable findings were observed following gross necropsies performed on three birds from the control and the 212, 468, and 948 mg ai/kg diet test concentrations at test termination.

This study **is scientifically sound** and is classified as **acceptable.**

Results Synopsis

Test Organism Size/Age (Mean Weight): 13.5 to 17.9 g / *ca.* 6 months old

LC_{50}: 948 mg ai/kg diet 95% C.I.: 758 to 1185 mg ai/kg diet

Endpoints Affected (based on reviewer's analysis of mortality and study author's analysis of all other endpoints): Mortality and body weight
Most Sensitive Endpoints (based on reviewer's analysis of mortality and study author's analysis of all other endpoints): Mortality

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based upon procedures outlined in OCSPP 850.2100 with modifications for 850.2200. The reviewer evaluated this study according to OCSPP 850.2200 and noted several deviations:

1. The physicochemical properties of the test substance were not reported.
2. Acclimation period conditions were not reported.
3. Temperature ranged from 20.1 to 25.1 °C whereas OCSPP 850.2200 guidance recommends 22 to 38°C. It should be noted that zebra finches may prefer a different temperature range than expressed in 850.2200 guidance.
4. The recommended humidity range based on OCSPP 850.2200 is 45-70%. In this study, humidity fell outside the recommended range (50 to 86%), but this is not considered a significant issue based on guidance in OCSPP 850.2200.
5. The photoperiod was 8 hours of light and 16 hours of darkness. OCSPP guidance recommends 14 hours of light. However, zebra finches may have different physiological responses to light, so the photoperiod deviation should be interpreted with caution.
6. The diet formulation used in the study contained a minimum of 14% crude protein. OCSPP guidance recommends that a range of 27 to 29% crude protein be provided to birds.

The deviations **do not** affect the acceptability of the study.

COMPLIANCE: Signed and dated Good Laboratory Practice (GLP), Quality Assurance, and Data Confidentiality statements were provided. The study was conducted in compliance with all pertinent U.S. EPA GLP standards (1989) and as accepted by the OECD Principles of GLP (1998) with the following exceptions: 1.) Characterization and stability of the test substance, 2.) preliminary, range-finding tests, and 3.) routine food and water analyses.

A. MATERIALS:

1. Test Material **Acetochlor Technical**

Description: Liquid

Lot No./Batch No. : 20180139 (Batch No.)

Purity: 96.6%

Stability of compound under test conditions: Stable. Measured concentrations of ambient Day 5 samples ranged from 95 to 99% of the Day -3 measured concentrations. Reviewer-calculated mean-measured test concentrations ranged from 85% to 97% of nominal.

Storage conditions of test chemicals: Ambient conditions.

Physicochemical properties of Acetochlor Technical.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism:

Species (common and scientific names): Zebra Finch (*Taeniopygia guttata*)
(EPA recommends using either bobwhite quail or mallard duck.)

Age at study initiation: *ca.* 6 months old
(EPA recommends: northern bobwhite: 10 - 14 days old, mallard: 5 days old)

Weight at study initiation (mean and range): 13.5 to 17.9 g

Source: International Pet & Supply Inc., South El Monte, CA

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study

Two acute oral range finding tests were conducted, one with two zebra finches and one with two canaries, at a dose of 2000 mg ai/kg administered via capsules. All birds regurgitated the test substance. Mortality was 50% (1 of 2) for zebra finches and 0% for canaries. A range-finding dietary probe was also conducted with three zebra finches dosed with test concentrations of 625 and 5000 mg ai/kg. The test resulted in no regurgitation but 100% mortality at the highest test concentration.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period:	7 weeks (pen acclimation)	
Conditions: (same as test or not)	Similar to test (details not provided)	
Feeding:	Transitioned from a commercially available finch food (Kaytee	

Parameter	Details	Remarks *Criteria*
	Forti-diet Pro Health Zebra Finch and golden sunburst millet sprays) to a pelleted diet (ZuPreem FruitBlend Flavor). Kaytee Hi Cal Grit was provided to aid with the birds' digestion. Water and feed were provided *ad libitum*.	
Health: (any mortality observed)	All birds were in good health at the start of the study. No mortalities were reported.	
Pen size and construction materials	Birds were housed in pens 29 x 26 cm with a ceiling height of 31 cm. External walls, ceilings, and floors were constructed of coated wire spaced 1.3 cm apart. Pens were separated by a fiberglass barrier.	Cages were equipped with perches and one cuttle bone. *Recommended pen size is about 35 x 100 x 24 cm*
Test duration	Exposure period – 5 days Post-exposure period – 3 days	 *Recommended test duration is 5 days with treated feed and at least 3 days observation with "clean" feed.*
<u>Test concentrations</u> nominal:	0 (negative control), 218, 500, 1000, 2000, and 4000 mg ai/kg diet	
measured:	<200 (<LOQ, control), 212, 468, 948, 1920, and 3419 mg ai/kg diet	*Five or six test concentrations should be used in a geometric scale, unless the LC_{50} > 5000 mg ai/kg diet.*

Parameter	Details	Remarks *Criteria*
Solvent/vehicle, if used type: amount:	 N/A N/A	 *Recommended solvents include distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. The solvent should not be more than 2%.*
Diet preparation and feeding	Diets containing the required amount of the test item were prepared by mixing the appropriate amount of the test item with the prescribed basal diet and 60 mL of acetone in a KitchenAid® stand mixer for approximately ten minutes. The control diet was prepared with basal ration and acetone only. The resulting diet was placed in labeled feed bags and periodically agitated until the acetone had volatized and was stored in a freezer until used.	 *The control group should be tested with a diet containing the maximum amount of vehicle used in treated diets.*
Feed withholding period	None specified	
Stability and homogeneity of test material in the diet determined (Yes/No)	Yes	
Number of birds per replicate/groups for negative control: for vehicle control: for treated:	 1 bird per replicate --- 1 bird per replicate	 *The recommended number of birds per replicate is a minimum of ten.*
Number of replicates/group (if used) for negative control: for vehicle control: for treated:	 10 --- 10	
Test conditions temperature: relative humidity (%): photoperiod:	 20.1 to 25.1 °C (average 23.2°C) 50 to 86% (average 70%) 8 hours light: 16 hours dark; approximately 468 lux of illumination	Ventilation of 15 room air volumes every hour. ***Recommended brooder temperature is** about 35°C (95°F)* ***Recommended room temperature is** 22-27°C (71-81°F)* ***Recommended relative humidity is** 30-80%* ***Recommended photoperiod** is a minimum of 14 hours of light.*
Reference chemical, if used	N/A	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks
Parameters measured (mortality/body weight/ mean feed consumption/ others)	- Mortality - Clinical signs of toxicity - Body weight - Food consumption - Necropsy	
Indicate the stability and homogeneity of test chemical in the diet	Stability: Results from diet storage stability analyses confirmed stability of the test substance in the basal diet at all test concentrations under ambient conditions for up to 5 days. Measured concentrations in ambient samples ranged from 97 to 99% of the Day -3 concentrations. Homogeneity: Homogeneity of the test substance in the diet was evaluated by collecting six samples each from the 218 and 4000 mg ai/kg. test diets at preparation on Day -3. Homogeneity samples were collected from the top, middle and bottom of the left and right sections of the mixing vessel. Measured concentrations in homogeneity samples taken from the lowest and highest test levels ranged from 86 to 98% of the nominal concentrations.	
Indicate if the test material was regurgitated	None reported.	
Treatments on which necropsies were performed	All mortalities and three surviving birds from the 212, 468, and 948 mg ai/kg diet treatment groups and the control.	There were no surviving birds at the 1920 and 3419 mg ai/kg diet treatment groups at test termination to be necropsied.

Parameters	Details	Remarks
Observation intervals	All birds were observed at least once daily for mortality, signs of toxicity, and abnormal behavior. Individual body weights were determined on Days 0, 1, 5, and 8. Average feed consumption values were determined daily for three days prior to the exposure period (Day -3 to Day 0), during the exposure period (Day 0 to Day 5), and daily for the post-exposure observation period (Day 5 to Day 8).	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

After 8 days, mortality was 0% in the control and the two lowest treatment groups. Mortality increased to 50% in the mean-measured 948 mg ai/kg diet treatment group and 100% in the 1920 and 3419 mg ai/kg diet treatment groups. Based on the nominal concentrations, the study author's 8-day acute dietary LC_{50} was 1000 (500 to 2000) mg ai/kg diet.

Table 3: Effect of Acetochlor Technical on Mortality of Zebra Finch (*Taeniopygia guttata*). [a]

Mean-Measured Concentration (Nominal) mg ai/kg diet		No. Birds/ Treatment	Cumulative mortality					
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 8
Negative Control		10	0	0	0	0	0	0
212 (218)		10	0	0	0	0	0	0
468 (500)		10	0	0	0	0	0	0
948 (1000)		10	0	0	0	1	3	5
1920 (2000)		10	0	1	5	7	10	10
3419 (4000)		10	0	7	8	10	10	10
NOAEC		500 mg ai/kg diet						
LC_{50} LD_{50}		1000 (500 to 2000) mg a.i./kg diet Not reported						
Reference chemical	Mortality	N/A						
	LC_{50}							
	NOAEC							

[a] Data obtained from Table 1, page 19 of the MRID.

B. SUB-LETHAL TOXICITY ENDPOINTS:

There were no statistically significant differences in mean body weight or mean body weight change for the birds in the 212 and 468 mg ai/kg diet test concentrations. There were statistically significant decreases in mean body weight change at the 1920 and 3419 mg ai/kg diet test concentrations from Day 0 to Day 1. There were also statistically significant reductions in mean body weight from Day 1 to 5 for surviving birds in the 948 mg ai/kg diet treatment group.

The mean feed consumption for the exposure period for the 212 and 468 mg ai/kg treatment groups was comparable to the control group. There was a concentration responsive reduction in mean feed consumption for 948, 1920, and 3419 mg ai/kg diet treatment groups during the exposure period. Post-exposure feed consumption for the birds in the 212 mg ai/kg diet test concentration was similar to the control group. Overall, no statistically significant reductions in food consumption were observed in any treatment group relative to the negative control group.

The mean daily dietary doses were 46, 95, 153, 153, and 132 mg a.i./kg bw/day for the 212, 468, 948, 1920, and 3419 mg ai/kg diet test concentrations, respectively.

Gross necropsies conducted on all mortalities resulted in observations of empty gastro-intestinal tracts, pale kidneys, mottled liver, pale liver, and pale spleens. No remarkable findings were observed following gross

necropsies performed on three birds from the control and the 212, 468, and 948 mg ai/kg diet test concentrations at test termination.

Table 4: Effect of Acetochlor Technical on Body Weight and Food Consumption of Zebra Finch (*Taeniopygia guttata*).

Mean-Measured Concentration (Nominal) mg ai/kg diet	Body Weight Change, g ± SD [a]		
	0 to 5 [b]	5 to 8	0 to 8
Negative Control	-0.5 ± 0.2	0.6 ± 0.2	0.2 ± 0.2
212 (218)	-0.3 ± 0.4	0.8 ± 0.3	0.6 ± 0.4
468 (500)	-0.4 ± 0.5	1.0 ± 0.8	0.6 ± 0.9
948 (1000)	-1.9 ± 0.9	1.8* ± 0.8	0.2 ± 0.6
1920 (2000)	-6.2 [c]	--	--
3419 (4000)	--	--	--

Mean-Measured Concentration (Nominal) mg ai/kg diet	Mean Food Consumption, g/bird/day ± SD [d]	
	Exposure Period Days 0 to 4	Post-Exposure Period Days 5 through 7
Negative Control	3.2 ± 0.3	3.4 ± 0.2
212 (218)	3.1 ± 0.5	3.5 ± 0.3
468 (500)	2.9 ± 0.4	3.8 ± 0.6
948 (1000)	2.2 ± 0.4	3.8 ± 0.5
1920 (2000)	1.1 ± 0.4	--
3419 (4000)	0.5 ± 0.2	--

-- = Could not be calculated due to mortality.
[a] Data obtained from Table 2, page 20 of the MRID.
[b] Calculated by the reviewer.
[c] Based on one bird due to significant mortality. Standard deviation could not be calculated.
[d] Data obtained from Table 3, page 21 of the MRID.
* Difference from the control group statistically significant at $p \leq 0.01$ (Bonferroni t-test).

C. REPORTED STATISTICS:

The LC_{50} value was calculated using non-linear interpolation and the 95% confidence interval was determined by the binomial test using the computer program of C.E. Stephan. Body weight data were compared by Bonferroni t-test using TOXSTAT®. Nominal concentrations were used for analysis and reporting.

LC_{50} (8 day): 1000 mg a.i./kg diet 95% C.I.: 500 to 2000 mg ai/kg diet

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Mortality data were analyzed using CETIS statistical software version 1.9.5.3 with database backend settings implemented by EFED on 7/25/17. Mean-measured concentrations calculated by the reviewer were used for analysis and reporting.

Due to the inability to obtain a valid 95% confidence interval with linear regression, the Untrimmed Spearman-Kärber method was used to estimate the LC_{50}.

LC_{50}: 948 mg ai/kg diet 95% C.I.: 758 to 1185 mg ai/kg diet

E. STUDY DEFICIENCIES:

No deficiencies were noted.

F. REVIEWER'S COMMENTS:

The reviewer's LC_{50} was lower than the study author's, however, this difference was likely attributed to the reviewer's use of mean-measured concentrations and the study author's use of nominal concentrations for analysis and reporting. Further, the study author used nonlinear interpolation to estimate the LC_{50} whereas the reviewer used the Untrimmed Spearman-Kärber method. The reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

Mean-measured concentrations were reviewer-calculated from ambient stability, verification, and homogeneity data.

The in-life portion of the test was conducted from September 19 to 27, 2019.

G. CONCLUSIONS:

This study **is scientifically sound** and is classified as **acceptable.** Mortality reached a maximum of 100% at the two highest test concentrations. The 8-day dietary LC_{50} (95% CI) was 948 (758 to 1185) mg ai/kg diet.

III. REFERENCES:

Stephan, C.E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, MN. Personal Communication.

Stephan, C.E. 1977. Methods for Calculating an LC50. Pages 65-84 in Aquatic Toxicology and Hazard Evaluations, American Society for Testing and Materials. Pub. No. STP 634. Philadelphia, PA.

West, Inc. and D.D. Gulley. 1996. TOXSTAT® Release 3.5 Western Ecosystems Technology, Inc. Cheyenne, Wyoming.

No other references were cited other than standard guidelines or methods.

OCSPP 850.2200 Sub-acute Avian Dietary Toxicity — **Eurofins EAG Agroscience, LLC**

Batch ID:	08-1677-9546	**Test Type:**	Sub-acute Avian Dietary Tox	**Analyst:**	
Start Date:	19 Sep-19	**Protocol:**	OCSPP 850.2200 Sub-Acute Bird	**Diluent:**	
Ending Date:	27 Sep-19	**Species:**	Taeniopygia guttata	**Brine:**	
Test Length:	8d 0h	**Taxon:**		**Source:**	International Pet Supply, El **Age:**
Sample ID:	04-3228-0305	**Code:**	51159004	**Project:**	Herbicide
Sample Date:	19 Sep-19	**Material:**	Acetochlor	**Source:**	Sharda Cropchem Limited
Receipt Date:		**CAS (PC):**		**Station:**	
Sample Age:	n/a	**Client:**	CDM Smith - E. Krupka		

PC Code 121601 MRID 51159004 mean-measured test concentrations
'08d Mortality Rate' endpoint...
Error with Log-Normal (Probit) Model:

The model requires two or more partial responses.

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	mg ai/kg	95% LCL	95% UCL	TU	S
03-9004-4987	08d Mortality Rate	Spearman-Kärber		LC50	948	758.3	1185		1

08d Mortality Rate Summary

Conc-mg ai/kg	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%
212		10	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%
468		10	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%
948		10	0.5000	0.1230	0.8770	0.0000	1.0000	0.1667	0.5270	105.41%	50.00%
1920		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	100.00%
3419		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	100.00%

08d Mortality Rate Detail

Conc-mg ai/kg	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
212		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
468		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
948		0.0000	1.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	1.0000	1.0000
1920		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
3419		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

OCSPP 850.2200 Sub-acute Avian Dietary Toxicity — **Eurofins EAG Agroscience, LLC**

Analysis ID:	03-9004-4987	**Endpoint:**	08d Mortality Rate	**CETIS Version:**	CETISv1.9.6
Analyzed:	31 Oct-20 1:40	**Analysis:**	Untrimmed Spearman-Kärber	**Status Level:**	1

PC Code 121601 MRID 51159004 mean-measured test concentrations
'08d Mortality Rate' endpoint...
Error with Log-Normal (Probit) Model:

The model requires two or more partial responses.

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.976791	0.048466	948	758.3	1185

08d Mortality Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-mg ai/kg	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	A/B	Mean	%Effect
0	N	10	0.0000	0.0000	0.0000	0.0000		0.0%	0/10	0	0.0%
212		10	0.0000	0.0000	0.0000	0.0000		0.0%	0/10	0	0.0%
468		10	0.0000	0.0000	0.0000	0.0000		0.0%	0/10	0	0.0%
948		10	0.5000	0.0000	1.0000	0.5270	105.40%	50.0%	5/10	0.5	50.0%
1920		10	1.0000	1.0000	1.0000	0.0000	0.00%	100.0%	10/10	1	100.0%
3419		10	1.0000	1.0000	1.0000	0.0000	0.00%	100.0%	10/10	1	100.0%

08d Mortality Rate Detail

Conc-mg ai/kg	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
212		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
468		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
948		0.0000	1.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	1.0000	1.0000
1920		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
3419		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Graphics